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METHODS FOR DETERMINATION OF CARBARYL RESIDUES IN FRUITS AND VEGETABLES

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Indian Standard

METHODS FOR DETERMINATION OF CARBARYL RESIDUES IN FRUITS AND VEGETABLES

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Indian Standard

METHODS FOR DETERMINATION OF CARBARYL RESIDUES IN FRUITS AND VEGETABLES

0. FOREWORD

0.1 This Indian Standard was adopted by the Indian Standards Institution on 24 May 1982, after the draft finalized by the Pesticides Residue Analysis Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Carbaryl formulations are extensively used in agriculture for the control of many insect pests of fruits, vegetables and other crops. Frequent and increased use of carbaryl formulations often result in harmful effects due to the toxic nature of residues. Careful assessment of residues is, therefore, an important step in safeguarding human health and in the establishment of sound regulatory policy.

0.3 In the preparation of this standard due consideration has been given to the limits of carbaryal residues which have been laid down under the provisions of Prevention of Food Adulteration Rules and the specified test methods are sensitive to the prescribed levels of residues.

0.4 This standard will enable the health authorities and others engaged in field to follow uniform test procedure for the estimation of carbaryl residues in fruits and vegetables.

0.5 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS : 2-1962*.

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1. SCOPE

1.1 This standard prescribes spectrophotometric and gas chromatographic methods for the determination of carbaryl (1-naphthyl-*N* methyl carbamate) residues in fruits and vegetables.

*Rules for rounding off numerical values (*revised*).

1.1.1 Spectrophotometric method may be adopted as a limit test for routine purposes and is generally applicable at the 0·1 ppm level, whereas Gas Chromatography shall be the reference method with the limit of detection at 0·02 ppm.

1.1.2 Though no set procedure for Thin Layer Chromatography (TLC) is being prescribed, Standardized TLC procedures may be followed, if necessary for the purpose of clean up, identification and confirmation of carbaryl residues.

2. SAMPLING

2.1 The representative samples for the purpose of estimating carbaryl residues in various fruits and vegetables shall be in accordance with the sampling procedures as prescribed in the relevant Indian Standards, wherever available.

3. PREPARATION OF SAMPLE

3.1 Apparatus

3.1.1 *Kuderna Danish Evaporative Concentrator* — 500 ml with 24/40 top joint and 24/25 bottom joint. Receiver ampoules, 10 ml capacity with 24/25 joint and retaining hooks.

3.1.2 *Waring Blender*

3.1.3 *Water-Bath*

3.2 Reagents

3.2.1 *Acetone* — analytical reagent grade, glass redistilled.

3.2.2 *Coagulating Solution* — prepared by dissolving 0·5 g of ammonium chloride (AR) in 400 ml of water containing 1 ml of orthophosphoric acid (AR).

3.2.3 *Diethylene Glycol Solution* — One percent (*v/v*) in glass redistilled methylene chloride.

3.2.4 *Hyflo Super-Cel*

3.2.5 *Methylene Chloride* — glass redistilled.

3.2.6 *Sodium Sulphate* — analytical reagent grade (anhydrous), powdered and granular.

3.3 Extraction — Transfer 50 g of sample to a blender. Add 150 ml of methylene chloride and 100 g of powdered anhydrous sodium sulphate.

Blend for two minutes at low speed and allow to settle for one minute. Decant the solvent into a Buchner funnel attached to a 500 ml filter flask and containing Whatman No. 1 filter paper covered with a thin coat of hyflo supercel or equivalent filter aid. Apply vacuum cautiously until all the solvent has filtered into the filter flask. Re-extract the sample with two 100 ml portions of methylene chloride and filter the extracts. Rinse with 50-ml methylene chloride.

3.4 Clean-up — Transfer the filterate (*see* **3.3**) to a 500 ml Kuderna-Danish evaporative concentrator and add 1 ml of diethylene glycol solution. Evaporate the solvent on a water bath. After the solvent has evaporated, rinse the evaporator with 5 ml of acetone from pipette and swirl. Transfer to 250 ml flask. Rinse the evaporator with additional acetone. Warm by swirling the flask gently in hot water for 30 seconds. Add 50 ml of coagulating solution and swirl the flask Allow the mixture to stand for 30 minutes, with occasional swirling. Filter using vacuum, through 3 mm layer of hyflo supercel on Whatman No. 1 in a Buchner funnel into a receiving flask. Wash the precipitate with 2 × 25 ml portions of acetone-water (1 : 9), allowing each washing to remain in contact with precipitate about 20 seconds before applying vacuum.

Transfer the filterate to a 250 ml separating funnel and add 25 ml of methylene chloride, shake well and allow the layers to separate completely. Drain the lower layer into a 250 ml Erlenmeyer flask. Repeat extraction of aqueous layer with 25 ml of methylene chloride and combine the extracts in the 250 ml Erlenmeyer flask. If the combined extracts are cloudy, add 5 to 10 g of anhydrous granular sodium sulphate and shake the mixture well. Decant the solution into a clean 250 ml Erlenmeyer flask and rinse the flask with small portions of methylene chloride. If the residue is expected to be greater than 2 ppm, the extract is diluted to a volume into a 100 ml volumetric flask and an appropriate aliquot is used.

4. SPECTROPHOTOMETRIC METHOD

4.1 Principle — The method is based on alkaline hydrolysis of carbaryl and colorimetric determination of the resulting 1-napthol with p-nitro-benzene-diazonium fluoborate as the chromogenic agent. A yellow complex is formed which is read spectrophotometrically at 475 nm.

4.2 Apparatus

4.2.1 *Spectrophotometer*

4.3 Reagents

4.3.1 *Acetic Acid* — analytical reagent grade.

4.3.2 *Carbaryl Reference Standard*

4.3.3 *Colour Reagent* — Prepared by dissolving 25 mg of *p*-nitrobenzene diazonium fluoborate in 5 ml methanol and then adding 20 ml acetic acid. Prepare fresh before use.

4.3.4 *Methanol* — analytical reagent grade, glass redistilled.

4.3.5 *Standard Alcoholic Pottassium Hydroxide Solution* — 0·1 N in methanol.

4.4 Estimation of Carbaryl Residues — Place the aliquot (*see* **3.4**) in an Erlenmeyer flask and add one ml of diethylene glycol solution and connect the flask to the column and evaporator. Evaporate the solvent as before. Rinse the walls of the flask down with 2 ml of the standard alcoholic potassium hydroxide solution from a pipette, rotating the flask to ensure complete contact. Allow the solution to stand for five minutes. Add exactly 17 ml of acetic acid and 1 ml of colour reagent with swirling. Allow the solution to stand exactly for one minute and determine the absorbance in a 1 cm cell using a spectrophotometer with a wavelength set at 475 nm against a reagent blank processed along with the sample. Determine the concentration of carbaryl from a curve obtained by plotting μg of the standard in ml against absorbance.

4.4.1 *Preparation of Standard Curve* — Weigh 50 mg of 1-naphthyl *N*-methylcarbamate (carbaryl), transfer to a 100-ml volumetric flask and dilute to a volume with methylene chloride. Take an aliquot of 10 ml and transfer to a 100 ml volumetric flask and dilute to volume with methylene chloride. Dilute the second solution in the same way and this gives a concentration of 5·0 μg/ml. Pipette aliquot of 0, 1·0, 3·0, 5·0 and 10·0 ml into a series of 500-ml Erlenmeyer flasks. Add 300 ml of methylene chloride to each of the flasks. Follow the same procedure of alkaline hydrolysis as described under **4.4**. Plot the absorbances of the standard solutions against μg of 1-napthyl *N* methylcarbamate to obtain a standard curve.

4.5 Expression of Results — Report carbaryl residues in the samples by using the standard curve in terms of parts per million.

5. GAS CHROMATOGRAPHIC METHOD

5.1 Principle — Carbaryl is hydrolyzed with alkali and the resulting 1-naphthol is reacted with pyridine and trichloroacetyl chloride to produce trichloroacetate which is measured by electron capture gas chromatography. The carbaryl content (ppm) is determined by comparing the response with the response for a known standard of similar concentration.

5.2 Reagents

5.2.1 *Hexane* — reagent grade, glass redistilled.

5.2.2 *Pyridine Solution* — Pass 50 ml of pyridine through a 10 mm i.d. liquid chromatographic column packed with florisil. Discard the first eluate that is used to wet the column. Dilute 0·10 ml of chromatographed pyridine to 100 ml with methylene chloride and store in a dark bottle.

5.2.3 *Florisil* — 60-100 mesh, activated at 110°C for 4 h.

5.2.4 *Trichloroacetyl Chloride Solution* — Dilute 0·1 ml of distilled trichloroacetyl chloride to 10 ml with methylene chloride in 10 ml volumetric flask.

5.2.5 *Sodium Hydroxide Solution* — 0·1 *N* in methanol.

5.3 Apparatus

5.3.1 *Gas Chromatograph* — A gas chromatograph equipped with ^{63}Ni electron capture detector is operated under the following suggested parameters. These parameters may be varied as per available facilities, provided standardization is done.

Column	Glass, 180 cm long and 0·4 cm i.d. packed with 10 percent DC-200 on 60-80 mesh gas chrom Q.
Column oven temperature	190°C
Injection port temperature	210°C
Detector temperature	230°C
Carrier gas	Nitrogen 75 to 100 ml/min
Recorder chart speed	1 cm/min

5.4 Procedure

5.4.1 Transfer suitable aliquot (*see* **3.4**) to a test tube, add 0·1 ml of polyethylene glycol or mineral oil and evaporate to dryness with a gentle stream of a air in a 40°C water bath. Add 0·2 ml of 0·1 *N* sodium hydroxide in methanol, rotate the tube to wet its sides with the mixture and shake for 5 minutes.

Evaporate the methanol, add 1 ml of the pyridine solution and heat the tube in an oil bath at 100°-105°C until the methylene chloride is evaporated. Remove the tube and cool in ice water, add one ml of the trichloroacetyl chloride solution, shake and again heat in the oil bath until the methylene chloride has evaporated. Remove the tube from oil

bath and add 10 ml of hexane, shake and add 5 ml of water and transfer the contents to a 60 ml separating funnel. Rinse the tube with 5 ml of hexane and transfer to the separating funnel. Shake the funnel for 30 seconds, drain the aqueous phase, add 5 ml of saturated sodium bicarbonate solution and shake for 15 seconds. Drain the sodium bicarbonate phase and wash the hexane layer with 5 ml of water. Drain the water layer and transfer the hexane layer to a volumetric flask by passing the hexane through a bed of anhydrous sodium sulphate. Wash the sodium sulphate with hexane and make the hexane solution, up to the mark. Inject a portion into the column using a microlitre syringe (5 µl injection). Identify the carbaryl peak by its retention time and measure the peak area.

5.5 Calculation

$$\text{Carbaryl (ppm)} = \frac{As}{Astd} \times \frac{Ats}{Atstd} \times \frac{M}{M_1} \times \frac{F}{F_1}$$

where

As = area sample;
$Astd$ = area standard;
Ats = attenuation sample;
$Atstd$ = attenuation standard;
M = mass, in µg of standard injected;
M_1 = mass, in g of sample;
F = final volume of sample in ml; and
F_1 = µl of sample injected.

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